EFFECTS OF BETAINE UPON THE CHOLESTEROL AND BILIRUBIN CONTENTS OF BLOOD PLASMA IN DIABETES MELLITUS*

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BEST and his co-workers in Toronto have shown that deposits of fat in animal livers caused by feeding of either fat or cholesterol may be made to disappear by the administration of choline. These workers have also shown that choline is not only curative but can prevent this deposition of fat. In the interpretation of such experiments, however, consideration must be given to a variety of modifying factors. Composition of diet, for example, is a factor. Casein was found to exert a lipotropic action; with diets of constant protein content the degree of fatty infiltration was found to vary with the fat content of the diets; and with diets of constant fat content the degree of fatty infiltration was found, in general, to vary inversely with the casein intake. (Different proteins have different lipotropic values. Gelatine, for example, has none). Pure sucrose caused an increase of fat, whereas the addition of protein prevented this excess deposition. Dried yeast and certain yeast preparations used for their contents of vitamin B_1 were found to be lipotropic, and on analysis were found to contain choline; "marmite" contained as much as 3 mg. per gram. Initial height of the glyceride content of the liver is to some extent another variable. A brief summary of the experiences of these authors will be found in a recent communication by Best and Channon.[1]

A study of any large group of diabetics shows that people suffering from this condition are no less liable to diseases of the liver (cirrhosis, malignancy, infection, etc.) than non-diabetics. Disease of the liver, secondary to disease of the gall bladder, is perhaps more common in the diabetic than in the non-diabetic. This is suggested from the finding, both in Joslin's clinic and in our own, that about one adult in every four had some disease of the gallbladder. Hæmochromatosis is definitely more common amongst diabetics than non-diabetics. Here, as is well known, the diabetes is, as a rule, a secondary and terminal manifestation. Aside from these conditions, however, there is much to suggest that there is a causal, as well as an accidental, relationship between disease of the liver and diabetes, as the following observations show.

Enlargement of the liver is common amongst juvenile diabetics. Priscilla White[2] found palpable livers in 40 per cent of her cases, and, in one case, the enlargement was such that the lower border was found in the pelvis.[3] That such enlargement is due essentially to fatty infiltration is suggested from the writer's experiences with a similar case.

A girl, 17½ years old, a diabetic of 7½ years' duration and a patient in this clinic since 1929, was admitted to the hospital into the service of Dr. C. P. Howard on February 4, 1935, in coma. The liver edge was then found 19 cm. below the costal margin in the mid-clavicular line. She recovered fully from the coma, and the subsequent course was uneventful, except for the rapid recession of the edge of the liver, which was at the costal margin when she was discharged from the hospital on February 25th, 21 days after admission. She was re-admitted about three months later (May 29th) again with severe acidosis, but not in coma, and, at this time, the liver edge was found 23 cm. below the costal margin in the mid-clavicular line. Again, following control of the diabetes, the liver edge receded rapidly, and when she was discharged from the hospital on August 5th it was at the costal margin. She was re-admitted to the hospital nine days later (August 14th), and the liver edge was then found 10 cm. below the costal margin. With treatment, it again receded and was at the costal margin on her discharge from the hospital on August 26th. When she returned to the Out-door Clinic for Diabetes on September 10th, the liver edge was again found 10 cm. below the costal margin.

It is of interest here to note that fatty infiltration of the liver is readily produced in the completely depancreatized animal, and, as Best *et al.*[4] have shown, the condition is greatly exaggerated, in spite of insulin therapy, when the choline content of the diet is kept low. Fatty infiltration of the liver is also a common post-mortem finding in the human diabetic in death due to diabetic coma. In fact, reference to fatty infiltration is one of the oldest observations on the pathology of diabetes; Mead[5] first drew attention to it in 1784.

In 1926, the writer[6] drew attention to the

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high incidence of excess quantities of bilirubin in the blood of diabetics; of a group of 130 cases hyperbilirubinæmia was found in 30—an incidence of 26.1 per cent. Since then de termination of the bilirubin content of the blood has been a routine test in this clinic; and in a later investigation of a much larger group of cases (500) by Dr. A. C. Corcoran the incidence of hyperbilirubinæmia was practically the same. In a more recent investigation, which included 3,000 analyses, the incidence of hyperbilirubinæmia—more than 0.5 units—was found to be 27.4 per cent. This is shown in the following Table which is a brief summary of a more extensive Table of another report.[7]

Units of bilirubin (range)	*Number*	*Percentage*
-0.5	2,178	72.6
0.6-1.0	699	23.3
1.1-1.5	76	2.5
1.6-2.0	19	0.63
2.1-	28	0.93

Assuming that, in the absence of biliary obstruction and hæmolysis, excess quantities of bilirubin in the blood are due to hepatitis, these data afford further proof of the high incidence of impairment of liver function in diabetes mellitus.

Further suggestive of an intimate relationship between liver function and carbohydrate tolerance in diabetes are the experiences with diets. Himsworth,[8] by well controlled experiments, has clearly demonstrated that diets rich in fat depress, whereas, diets rich in carbohydrates improve, carbohydrate tolerance; and that diets rich in fats decrease, whereas, diets rich in carbohydrates increase, the sensitivity of the individual (animal or man) to insulin. From the observations of Himsworth it would appear that the explanation of these phenomena is to be found in the liver; but, perhaps, the most impressive evidence of the importance of the liver in the control of diabetes is the finding of Soskin, Allweiss and Cohen.[9] These authors have shown that if the blood sugar is kept at the normal level by administration of insulin it is possible to obtain perfectly normal blood sugar time curves in the completely depancreatized animal, *providing the liver is not disturbed.*

In view of the above observations, an attempt was made to determine the value of one of the derivatives of choline—betaine—in a group of diabetics who failed to respond to treatment in the usually expected manner with our high carbohydrate-low calorie diet.

As has been shown repeatedly by the writer, the high carbohydrate-low calorie diet which has been in use in this clinic for more than six years[10] leads, in the majority of cases, to marked improvement of carbohydrate tolerance,[11] and the experiences, in general, fit in with the above-mentioned observations of Himsworth. Like all other diets used in the treatment of diabetes, however, this diet has its failures. A careful study of these failures showed that, in the majority of cases, treatment had little or no effect upon the cholesterol content of the blood, whereas, as the writer has shown repeatedly[12] one of the most striking characteristics of the high carbohydrate-low calorie diet is an immediate and sustained decrease of plasma cholesterol. With this diet, also, as with the older methods of treatment,[13] it has been noted that the patients whose blood cholesterol were not normal have not done as well as those whose bloods contained normal quantities of cholesterol. Minor dietary indiscretions resulted in more prolonged hyperglycæmia; the insulin dosages were higher; and mild infections (colds, etc.) were more common and led to more prolonged hyperglycæmia than with similar conditions, but with normal amounts of cholesterol in the blood. Digestive disturbances commonly met with in the past with the high fat diets have also been noted with the new diet when the bloods contained excess quantities of cholesterol. Of particular interest here is the fact that in the majority of these failures the bloods not only contained excess quantities of cholesterol but also of bilirubin. The possibility, therefore, occurred to the writer that in these cases *constant exposure to excess quantities of cholesterol in the blood may have the same effects upon the liver in the human being as those which were found by Best and his co-workers in animals after feeding of cholesterol.**

An obvious difficulty in putting this idea to the test of experiment is that, unlike in animal

* Both Dr. L. J. Rhea and Dr. J. E. Pritchard, our pathologists, have been impressed with the fact that more recently at autopsies of diabetics they have not found the fatty livers which were common prior to the use of the high carbohydrate diet. Of interest here is also the report of a case of typhoid fever in a child of 8 years with marked enlargement of the liver due to excess cholesterol esters.[14]

experiments, the effects of cholesterol upon the liver cannot be determined directly; measurement of the size of the liver is of little or no value, since anatomical integrity and functional efficiency are not necessarily, and as a rule are not, synonymous. The only other evidence, clinically, are the results of tests of liver function and, as yet, these are not very satisfactory. Bollman and Mann[15] have shown that when liver tissue is reduced in animals by repeated lobectomy the first indication of liver failure is accumulation of uric acid in the blood. From other data it would also appear that uric acid is dealt with exclusively by the liver. Theoretically, therefore, it would appear that the effects of betaine feeding would best be observed by analysis of this blood constituent. Actually, however, from our own experiences with many thousands of tests, we have found the estimation of uric acid a very unsatisfactory test of liver function. The reserve capacity of the liver,[16] its ability to regenerate,[17, 18] and the fact that failure of one function does not necessarily imply failure of another, are some of the difficulties in the interpretation of data. This applies also to all of the other tests of liver function (galactose, lævulose, the phthalein tests, etc.). From our own experiences, at least, the van den Bergh reaction appears to be the most satisfactory qualitative index of early impairment of liver function. In this study, therefore, the effects of betaine feeding were measured by determination of the bilirubin content of the blood. The cases which were selected for this study included only those whose bloods not only contained excess quantities of cholesterol but also excess quantities of bilirubin.

In this report are recorded the experiences with ten diabetics, each of whom had been under observation for at least one year before and at least one year after treatment with betaine. No cases are included in which there were less than ten determinations of cholesterol and ten of bilirubin both before and after treatment. The betaine used was obtained from the Research Laboratories of the Eastman Kodak Company, Rochester, N.Y. (betaine hydrochloride—P. 345). In each case, the dosage was 0.5 gram, three times a day. No disturbing effects were noted with the drug at any time. The combined data are shown in the accompanying Table.

EFFECTS OF BETAINE FEEDING UPON CHOLESTEROL AND BILIRUBIN CONTENTS OF BLOOD PLASMA IN TEN CASES OF DIABETES MELLITUS

Cholesterol
(mgms. per 100 c.c.)

Remarks		*Period**	
		Before†	*After†*
Number of analyses		113	166
Cholesterol:	maximum	432	299
	minimum	151	161
	average	258	244
	σ	122	118
	PE(m)	7.71	6.13
	PEΔ	9.8	
	$\frac{\Delta}{PE\Delta}$ =	1.4	

Bilirubin
(Units)

Remarks		*Period**	
		Before†	*After†*
Number of analyses		113	178
Bilirubin (units)	maximum	2.4	2.5
	minimum	0.2	0.2
	average	0.86	0.49
	σ	0.28	0.32
	PE(m)	1.77	1.61
	PEΔ	2.4	
	$\frac{\Delta}{PE\Delta}$ =	15.0	

*Cases under observation for less than one year are not included.

†Cases with less than ten blood analyses in each period are not included-

σ = Standard Deviation.

PE(m) = Probable Error of Mean.

PEΔ = Probable Error of Difference between Means.

$\frac{\Delta}{PE\Delta}$ = Ratio of Difference between Means to Probable Error of Difference.

In order to simplify demonstration of the Ratio in the cases of bilirubin, the number of units is multiplied by 100.

It will be observed that the average concentration of cholesterol in the blood plasma after administration of the betaine was slightly less than that noted during the control period. However, when these average values are judged by their probable errors it is obvious that the difference found is of little or no significance, since the ratio of the difference to its probable error was 1.4 only. This does not, however, necessarily imply that the betaine was inert. In the interpretation of these data consideration must be given to the fact that the cholesterol content of the blood may not be a proper

index of the degree of deposition of fat in the liver. It should here be observed that when fatty infiltration of the liver was produced by Best and his co-workers in their diabetic dogs the condition was not reflected in the blood; there was no increase of blood fat. Another important observation of these authors was that though the choline administered led to disappearance of fat from the fatty livers, regardless of the method of their production (cholesterol or fat feeding) it did not have the same effect upon the cholesterol esters as upon the other lipoids. (This result was due in part to the large amount of cholesterol fed [1 to 2 per cent of diet]. Using 0.2 per cent, the effect of choline on the esters is much more marked.)*

That the betaine was not inert in the above-mentioned diabetics is suggested from its effects upon the bilirubin contents of the blood. It will be observed that in the 113 tests during the control period the average amount of bilirubin in the blood was 0.86 units, whereas, after administration of the betaine it was 0.49 units—a reduction of approximately 57 per cent. That the difference noted was significant is clearly shown by the ratio of the difference to its probable error $\frac{\triangle}{PE\triangle} = 15.0$. These data, therefore, justify the conclusion that the feeding of betaine in these cases resulted in improvement of liver function, at least so far as the metabolism of bilirubin is concerned. This conclusion is, of course, statistical. It, therefore, may, or may not, and need not necessarily, apply to an individual.†

From the combined data the writer has the impression that there has also been some improvement of carbohydrate tolerance, judging from the increase of carbohydrates and reduction of insulin dosages; and that the improvement paralleled improvement of liver function, judging from the reduction of the bilirubin contents of the blood. However, an impression is not proof and a much longer period of observation will be necessary, in view of the gain of carbohydrate tolerance generally noted with the high carbohydrate-low calorie diet.[11] The data are, however, very suggestive, in view of the fact that these cases were selected for this study because they were regarded as failures in their response to treatment.

It is of interest here to note that the average protein content of the high carbohydrate-low calorie diet is appreciably higher than that of the older diets of much higher fat and much lower carbohydrate contents. An interesting speculation, therefore, is whether, aside from carbohydrates improving and fats depressing carbohydrate tolerance, as shown by Himsworth, some of the improvement noted with this diet may not be due to its protein content, in view of the observation by Best and his co-workers that some dietary proteins contain an effective lipotropic factor. Should this be found to be so, it is obvious that, in the future, in the construction of diabetic diets consideration will have to be given not only, as in the past, to carbohydrates, fat, protein, etc., but, also, to the content of choline and its derivatives. The definitely proved harmful effects of cholesterol, and the tendency of certain fats to cause fatty infiltration more readily than others, may also have to be considered. Best (personal communication) for example, has found that beef dripping was more effective in producing fatty livers than butter. The experiences reported here, therefore, certainly warrant further investigation of the effects of betaine in human diabetes, especially in view of the fact that no harm was observed with the betaine, and that, compared with the dosage of choline which were used in the experimental animal, the amounts of betaine used in the above-mentioned cases were small.

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* Personal communication.

† It is of interest here to note that in their early experiments with fatty livers in animals, produced by feeding of fat and cholesterol, Best and his co-workers found large amounts of bile pigment in the urines, which disappeared after administration of choline. No tests were made of the bilirubin contents of blood. (Personal communication).

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THE UNSTABLE COLON*

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IT is an interesting but none the less regrettable fact that the medical profession generally does not regard purely functional disorders as worthy of serious attention. For centuries, the profession has been trained to believe that the smallest departure from normal function must be due to some objective pathological change which needs only a sufficient number of clinical and laboratory tests to demonstrate its presence. This tradition has become deeply entrenched in our medical teaching. The colon has suffered more than its share as a result of this medical apathy. We are content for the most part to accept the patient's diagnosis of chronic constipation, prescribe a high roughage diet with some moderately irritating laxative, and hope that the next time the patient comes he will have some really interesting complaints.

Within recent years, however, our knowledge of the physiology of the colon has increased materially. We know that this organ *has* useful functions to perform, if we will only allow it to perform them, that it is not the source of toxins which are readily absorbed and produce serious chronic diseases. We also know that the normal natural functioning of the colon is essential to continued good health and well-being. Therefore I think that the functional disturbances of the colon are well worthy of our interest and attention.

ANATOMY AND PHYSIOLOGY

We learn from comparative anatomy that man has an unusually short uncomplicated digestive tract, and we have abundant proof that only late in his development did he learn to use the products of the soil—primarily he was a hunter and fisherman. These anatomical facts indicate clearly that man's digestive tract was not designed for a diet containing excessive roughage.

The known functions of the colon are the absorption of water, the digestion of cellulose, the secretion of mucus, and the propulsion of its content from cæcum to rectum. The digestion of cellulose is a process of fermentation aided by bacterial action, and therefore it requires much more time than the rapid chemical reactions of digestion in the small intestine. The greater width of the colon and its sluggish activity are admirably adapted for this purpose, as for the absorption of water. We know from many experimental observations that these functions are carried out in the proximal colon, and that normally, this portion always contains food residues which by the time they reach the descending colon are practically dry and their bacterial content dead. Therefore if the colon is to carry out its proper functions the cæcum, ascending colon and transverse colon *are never empty*. This important point has an obvious bearing on the effects of laxatives on colon function.

Another point which has some bearing on our discussion concerns the innervation of the colon. The extrinsic nerve supply is double, through the sympathetic and para-sympathetic systems. We know that stimuli from the higher centres and cortex, and that conditioned reflexes are mediated through these nerves, but, unfortunately, we do not yet know the interrelation of the two systems. It was formerly

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